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The frequency of co-positivity of anti-smooth muscle antibody and anti-nuclear antibodies and their contribution to the diagnosis of autoimmune hepatitis

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ABSTRACT

Aim: Autoimmune hepatitis (AIH) is a chronic disease observed especially in women. The International Autoimmune Hepatitis Group recommends scoring systems for diagnosis using clinical and laboratory data. All scoring systems gave points to autoantibodies as anti-nuclear antibody (ANA) and anti-smooth muscle antibody (SMA) positivity. This study investigates the impact of the co-positivity of the ANA and SMA antibodies on the autoimmune hepatitis diagnosis.

Material and Method: We monitored 78 autoimmune liver disease (autoimmune hepatitis, AIH) suspected patients with positive SMA antibody and then further tested for ANA between 2014 and2021. SMA test was screened at 1/40 and 1/100 titers and patients who were positive were taken to further dilution. The ANA test was screened at a titer of 1/40 and 1/160, a positive result was found to be repeated with advanced dilutions. All patients' autoantibody scores of simplified AIH diagnostic system were calculated.

Results: Seventy eight patients with positive SMA antibodies screened for ANA test with 1/40 and 1/160 titer, only 2 patients was found to be negative. The most frequently observed ANA pattern is cytoplasmic linear fibrils (68%). The 95% ANA positive results was examined at a screening titer of 1/160. The 95% SMA positive results was found at a screening titer of 1/100. The autoantibody scores of 76 patients were +2, patient's scores were +1.

Conclusion: SMA antibody positivity is accompanied by a high rate of ANA antibody positivity but the co-positivity didn't effect diagnostic score systems. On the other the co-positivity could be a sign of another associated autoimmune diseases.

Keywords: Anti-smooth muscle antibody, anti-nuclear antibodies, autoimmune hepatitis

INTRODUCTION

Autoimmune hepatitis (AIH) was first identified as chronic hepatitis in young women in 1951 and was characterized in the USA a short time later (1, 2). In 1956, by discovering its association with anti-nuclear antibodies (ANA), lupoid hepatitis was created (3). The emergence of immuno-fluorescence assay (IFA), radio-immunno assay method (RIA), enzyme-linked immunosorbent assay (ELISA), molecular methods, and cloning techniques allowed the identification of hepatocellular auto antigen in AIH (**Table 1**). Characterizing the humoral and cellular immune systems in patients and animal models of autoimmune liver disease has improved knowledge (1, 4-7).

IAH is divided into two main groups:

i. Type 1 AIH (AIH-1); related with anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (SMA) positivity.

ii. Type 2 AIH (AIH-2); related with anti-liver kidney microsomal antibody type 1 (anti-LKM1), anti-LKM3 and/or anti-liver cytosol antibody type 1 (anti-LC1) positivity.

Both genetic and environmental factors are thought to be influential in etiology. An immune response targeting liver autoantigens is believed to initiate and sustain liver damage (1, 7).

Various scoring systems prepared by the International Autoimmune Hepatitis Group are used to diagnose autoimmune hepatitis. The most commonly "revised Scoring System" and "simplified scoring system" used. Both scoring systems gave points to ANA and SMA positivity (8).

1-Revised International Autoimmune Hepatitis Group Modified Scoring System:

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The revised original scoring system is a diagnostic method to ensure the systematic evaluation of patients. This scoring system was based on 12 clinical components, originally used developed as a tool for scientific purposes. Though the revised original diagnostic criteria were incorporated into clinical diagnosis of AIH, it is a very complex score system, and even including a variety of parameters of questionable value, it is difficult for wider applicability in routine clinical practice.

ANA, SMA, and LKM autoantibodies. On the diagnosis of autoimmune hepatitis when the total reaches ≥17 points (8).

2- To simplify the use of revised original diagnostic scoring system, the IAIHG defined simplified diagnostic criteria for routine clinical practice in 2008. The simplified score system is a reliable and simple tool to establish and exclude the diagnosis of AIH more frequently in liver diseases concurrent with immune manifestations, it was purely meant for clinical purposes. The simplified score system has superior specificity and accuracy comparing to the original revised scoring system, but only includes four clinical components, and no treatment response in the scoring system, it is generally accepted that simplified score system has a lower sensitivity (**Table 1**) (8).

Table 1. The simplified AIH diagnostic score system (*Sum of points achieved for all autoantibodies (maximum 2 points))

	Clinical feature	Results	Scores
1	ANA or SMA	≥1:40 by IIF	+1
	ANA or SMA	≥1:80 by IIF	+2*
	Anti-LKM1 (alternative to ANA and SMA)	≥1:40 by IIF	+2*
	Anti-SLA (alternative to ANA, SMA and anti-LKM1)	Positive	+2*
2	IgG	>UNL	+1
		>1.1 UNL	+2
3	Liver histology	AIH	+1
		Typical AIH	+2
		Atypical AIH	0
4	Absence of viral hepatitis	Yes	+2
		No	0
Total scores		≥6: probable AIH ≥7: definite AIH	

This study investigates the co-positivity of the ANA and SMA antibodies used to diagnose autoimmune hepatitis and their contribution to the diagnosis of autoimmune hepatitis.

MATERIAL AND METHOD

The study was carried out with the permission of Atadek, Acibadem University Medical Faculty Clinical Researches Ethics Committee (Date: 2022, Decision No: 19-05). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Clinical Sample Selection

From fifty-one (51) different primary, secondary and tertiary health care centers in seven geographical regions of Turkey, blood samples are sent to our central clinical laboratory (Acibadem Labmed, Istanbul, TR) for ANA and SMA testing. All ANA and SMA analyzes were performed in the central microbiology laboratory. The selection of patients, testing procedures, and follow-up period decayed for eight years between 2014 and 2021.

This study included 78 patients monitored for suspected autoimmune liver disease, with positive SMA antibody and ordered for an ANA test.

ANA and SMA test studies

SMA tests were studied using the RLKs–Rat wrapped (Rat/Monkey) (Aesku, Wendelshei, Germany) kit on a Helmed IFA systems (Aesku) device using the IFA method. The slides were evaluated by two IIFA microscopist (one experienced laboratory technician and microbiology specialist) under a Led Immunofluorescence Microscope (Motic, Hong Kong). In case of incompatibility between the two readings, the SMA test was repeated with the Euro plus LKS Mosaic kit (Euroimmun, Lübek, Germany) the patients who were screened at 1/40 and 1/100 titers, and positive patients were diluted at 1/320, 1/1000, and 1/3200 titers.

The ANA tests were performed using Helios automated IFA systems (Aesku, Wendelsheim, Germany) and HEp-2 Standard kit (Factory, Country?). The images of the ANA slides were taken using Helios automated IFA systems, added to the report through a Laboratory Information System (LIS), and then stored. In October, two IIFA microscopist (one experienced laboratory technician and microbiology specialist) examined the slides using a Led Immunofluorescence Microscope (Motic, Hong Kong). In the case of discordance between two readings, an ANA test was repeated using Mosaic HEP20-10/Liver (Monkey) (Euroimmun, Lüebeck, Germany). The patients who were screened at titers of 1/40 and 1/160 and positive were diluted at titers of 1/320, 1/1000, and 1/3200.

Simplified AIH diagnostic score system

All patients' simplified AIH diagnostic system scores were sorted from their files.

RESULTS

The demographic analysis of 78 patients is summarized in **Table 2**.

Table 2. Demographic information of the patients'

	SMA Positive (n: 78)
Gender	Female n:65 (83.33%)
Age, years	46.8 (15-89)

(Abbreviations: SMA: Smooth muscle Antibody)

The ANA results of SMA-positive patients (pattern and titers) are presented in **Table 3**. Cytoplasmic linear fibrillary pattern was found to be positive most frequently (68%). After that, homogeneous (13.2%), cytoplasmic reticular (7.5%), spotted (2.5%), and nucleolar (1.3%) were found, respectively (**Figure 1**).

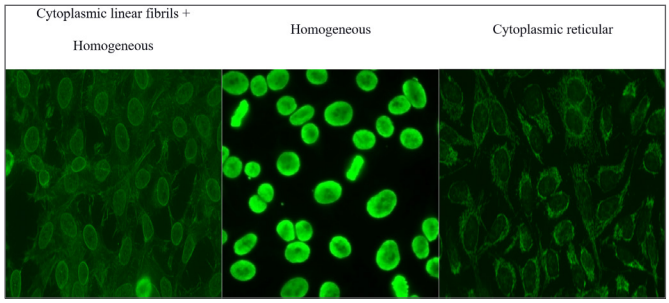

Figure 1: The captured pictures of 3 most common ANA patterns.

Table 3. Distribution of SMA-positive patients and their ANA patterns at a titer of 1/160. (Abbreviations: ANA: Anti-Nuclear antibody, SMA: Smooth muscle Antibody)

ANA Patterns	Number and percentage of patients
Cytoplasmic linear fibrils	53 (68.0%)
Homogeneous	10 (13.2%)
Cytoplasmic reticular	6 (7.5%)
Negative	6 (7.5%)
Speckled	2 (2.5%)
Nucleolar	1 (1.3%)

The patient, whose 6 ANA tests were negative, repeated the ANA test at a titer of 1/40 again. Homogeneous cytoplasmic linear fibrillary patterns were observed in 3 patients, linear fibrillary patterns were observed in 1 patient, and two patients were found to be negative at both titers.

In the ANA screening test of 78 SMA-positive patients performed at a titer of 1/160, 72 were found positive and received +2 points from autoantibodies score of simplified AIH diagnostic system. Six patients whose ANA test was negative, the ANA test was repeated at a titer of 1/40, and 4 patients were found positive and received +1 points from autoantibodies score of simplified AIH diagnostic system. Of the patients with a positive SMA test, 76 were positive at 1/100 titer positive and received +2 points from autoantibodies score of simplified AIH diagnostic system. And two were positive at 1/40 titer positive and received +1 points from autoantibodies score of simplified AIH diagnostic system (**Figure 2**) (**Table 4**).

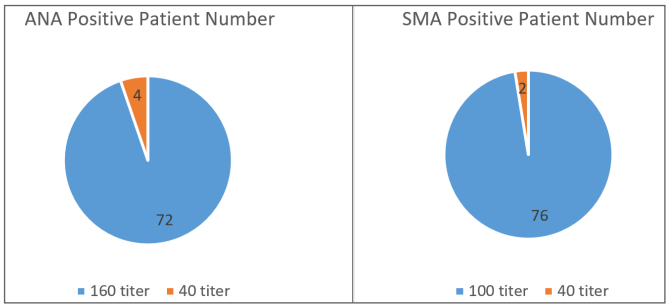

Figure 2: Titers of SMA and ANA tests positive. Abbreviations: ANA: Anti-Nuclear antibody, SMA: Smooth muscle Antibody (1:160, 1:40, 1:100 titers).

Table 4. Distribution of co-positivity of ANA- SMA and autoantibodies scores of simplified AIH diagnostic score system.

SMA/ ANA Screening Titers	Number and percentage of patients	simplified AIH diagnostic score (points achieved for all autoantibodies)
SMA positive 1/100 ANA positive 1/160	72	+2
SMA positive 1/100 ANA positive 1/40	2	+2
SMA positive 1/100 ANA negative	2	+2
SMA positive1/40 ANA positive1/40	2	+1

Distribution of the co-positivity, their titers, number of patients and autoantibodies score of Simplifed AIH diagnostic system were summarized at **Table 3**.

We calculated autoantibodies scores of simplified AIH diagnostic system. Scores received from single antibody couldn't increase by co-positivity.

DISCUSSION

Autoimmune hepatitis is a disease of exactly unknown cause that occurs in women of all ages and races. The diagnosis is made according to laboratory criteria, including clinical and specific autoantibodies (9, 10, 11). Autoimmune hepatitis (AIH) is an immuno-inflammatory liver disease with a non-self-limiting clinical course in which immunosuppressive agents are required in most affected patients (1, 12).

In recent years, the molecular targets of most autoantibodies-related associated diseases have been identified and characterized. The recent autoimmune disease diagnostic criteria clarified the place of autoantibodies in the diagnosis (13, 14).

Scoring systems including various clinical and laboratory data are used to diagnose autoimmune hepatitis. The "revised Scoring System" and the "simplified scoring system" prepared by the International Autoimmune Hepatitis Group are frequently used. ANA, SMA, LKM, and SLA are the autoantibodies used in scoring (1, 15).

It is common more than one autoantibody to appear together simultaneously in autoimmune diseases.

More than one autoantibody may also coincide in autoimmune hepatitis disease. 20-40% of the patients with AIH had another associated autoimmune or auto inflammatory disease (concomitant autoimmune diseases (CAIDs) (16).

Gergenli et al. (17) found an association in 35% of the patients, 12% had vitiligo, 6% had celiac disease, 6% had juvenile idiopathic arthritis, 6% had Familial Mediterranean Fever (FMF), and one patient had both type-1diabetes mellitus and Hashimoto thyroiditis (HT) .

Gökçe et al. (18) represented a case with atypical celiac patient with AIH. The patients had SMA + celiac autoantibodies and gave rapid responses to treatment.

Gencdal et al. (19) recommend to check AIH patients for celiac. 8.7% of patients in the AIH group were serologically and histologically diagnosed with celiac disesase.

Ordering multiple autoantibody tests used for diagnostic scoring together in patients with suspected autoimmune hepatitis, and their co-positivity is often observed (8).

The ANA international consensus pattern (ICAP: a sub-division of the American College of Rheumatology (ACR)) and the European Autoimmunity Standardization Initiative/immunology Union of international associations (EASI/IUIS) reached a consensus on reporting the ANA patterns. In contrast, for ANA cytoplasmic/mitotic apparatus patterns do not imply a clear position on the reporting ANA as negative or positive result (20-24).

In our study, of the 78 SMA-positive patients, 76 tested positive in the ANA test. Of the 76 ANA pattern positives, 75% are related to cytoplasmic patterns. The co-positivity of ANA and SMA were high but the co-positivity didn't effect diagnostic score systems, since sum of points achieved for all autoantibodies restricted to maximum 2 points. On the other the co-positivity could be a sign of another associated autoimmune or auto inflammatory disease (concomitant autoimmune diseases (CAIDs)).

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Atadek, Acibadem University Medical Faculty Clinical Researches Ethics Committee (Date: 2022, Decision No: 19-05).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: The author declare that she have participated in the design, execution, and analysis of the paper, and that she have approved the final version.

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